REMARKS

Claims 1-40 are pending. Applicants gratefully acknowledge the courtesy of the Examiner during the interview held May 7, 2002.

The amendments are supported by the original disclosure and, thus, no new matter has been added. If the Examiner should disagree, however, she is respectfully requested to point out the challenged limitation with particularity in the next Action so support may be cited in response.

Support for the addition of "which may contain" in the initial recited step of the method and "determining whether the total nucleic acid in said sample is higher or lower than a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg" in the last recited step of the method can be found implicitly on page 1 of the specification in the first paragraph following the "Background of Invention" heading. The kit claims have been amended by adding a positive control for the threshold amount of contamination as shown in the Examples.

35 U.S.C. 103 – Nonobviousness

Claims 1-3, 6-12, 14-25, 28-32 and 34-37 were rejected under Section 103(a) as allegedly being unpatentable over Hartley (US 5,043,272) in view of Eberle et al. (US 5,413,906). Applicants traverse.

Claims 4-5, 13, 26-27, 33 and 39-40 were rejected under Section 103(a) as allegedly being unpatentable over Hartley (US 5,043,272) in view of Wu et al. (Genomics 4:560-569, 1989) and Respess (US 5,599,662). Applicants traverse.

Claim 38 was rejected under Section 103(a) as allegedly being unpatentable over Hartley (US 5,043,272) in view of Kozlowski et al. (US 6,096,499). Applicants traverse.

As was explained during the interview, Applicants' claimed invention is directed to a method of increased sensitivity for determining levels of contamination in a sample. Hartley's PCR reaction was not used quantitatively and there was no suggestion that such method would amplify starting amounts of nucleic acid in a quantitative manner. This failure of the Hartley reference is not remedied by the disclosures of the other cited references.

Modifying Hartley's PCR reaction with random hexamer primers into a quantitative method would not have been motivated by the disclosures of the other cited references. There is neither teaching nor suggestion in those other cited references to use Hartley to amplify low amounts of substrate before a quantifying method, and subsquently to be able to determine the absolute quantity of the original amount of nucleic acids at the level of sensitivity recited in the claims.

Moreover, the cited references fail to teach a quantitative method that does not depend on knowledge of the sequences of the nucleic acids in the sample and is able to assay complex mixtures of nucleic acids. These factors are important because the claimed invention requires measurement of the total amount of nucleic acids in the sample irrespective of their sequences or the complexity of nucleic acids in the sample.

Furthermore, there is no indication in any of the cited references that the claimed invention could have been made as proposed in the Office Action with a reasonable expectation of success. The cited references neither teach nor suggest a sensitivity of detection in the range of 100 pg when amplification precedes quantification. In this respect, there has been no showing that Hartley's method would have amplified the initial amount of nucleic acids in a manner to allow quantitation.

Withdrawal of the Section 103 rejection is requested because the invention as claimed would not have been obvious to a person of ordinary skill in the art at the time it was made.

Having fully responded to all of the pending objections and rejections contained in the Office Action (Paper No. 17), Applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information is required.

Respectfully submitted,

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APPENDIX
MARKED-UP VERSION TO SHOW CHANGES

IN THE CLAIMS

The claims are amended as follows.

1. (3x Amended) A method for determining [measuring] total nucleic acid in a sample, which comprises:

a) mixing at least one random primer at least 4 nucleotides in length, having at least one detectable species with a sample which may contain nucleic acid,

b) adding at least one nucleotide triphosphate having at least one binding species and optionally at least one second nucleotide triphosphate,

c) adding at least one nucleic acid polymerase,

d) incubating the mixture of step c), under conditions which allow said at least one nucleic acid polymerase to be active,

e) contacting the mixture of step d) with at least one solid phase,

f) measuring total nucleic acid in said sample by measuring the total amount of said at least one detectable species bound to said solid phase, and

g) determining whether the total nucleic acid in said sample is higher or lower than a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg.

2. (3x Amended) A method for determining [measuring] total nucleic acid in a sample, which comprises:

a) mixing at least one random primer at least 4 nucleotides in length, having at least one binding species with a sample which may contain nucleic acid,

b) adding at least one nucleotide triphosphate having at least one detectable species and optionally at least one second nucleotide triphosphate,

c) adding at least one nucleic acid polymerase,

d) incubating the mixture of step c), under conditions which allow said at least one nucleic acid polymerase to be active,

e) contacting the mixture of step d) with at least one solid phase,

f) measuring total nucleic acid in said sample by measuring the total amount of said at least one detectable species bound to said solid phase, and

g) determining whether the total nucleic acid in said sample is higher or lower than a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg.

3. (3x Amended) A method for determining [measuring] total nucleic acid in a sample, which comprises:

a) mixing at least one random primer at least 4 nucleotides in length with a sample which may contain nucleic acid,

b) adding at least one nucleotide triphosphate having at least one binding species and optionally at least one nucleotide triphosphate having at least one detectable species and optionally at least one second nucleotide triphosphate,

c) adding at least one nucleic acid polymerase,

d) incubating the mixture of step c), under conditions which allow said at least one nucleic acid polymerase to be active,

e) contacting the mixture of step d) with at least one solid phase,

f) measuring total nucleic acid in said sample by measuring the total amount of said at least one detectable species or the amount of said at least one binding species bound to said solid phase, and

g) determining whether the total nucleic acid in said sample is higher or lower than a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg.

4. (3x Amended) A method for determining [measuring] total nucleic acid in a sample, which comprises:

a) mixing at least one first labeled random primer at least 4 nucleotides in length having at least one binding species and at least one second random primer at least 4 nucleotides in length having at least one detectable species, with a sample which may contain nucleic acid,

b) adding at least one nucleic acid ligase,

c) incubating the mixture of step b), under conditions which allow said at least one nucleic acid ligase to be active,

d) contacting the mixture of step c) with at least one solid phase,

e) measuring total nucleic acid in said sample by measuring the total amount of said at least one detectable species or the amount of said at least one binding species bound to said solid phase, and

f) determining whether the total nucleic acid in said sample is higher or lower than a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg.

5. (3x Amended) A method for determining [measuring] total nucleic acid in a sample, which comprises:

a) mixing at least one first labeled random primer at least 4 nucleotides in length having at least one binding species and at least one second random primer at least 4 nucleotides in length having at least one detectable species, with a sample which may contain nucleic acid,

b) adding at least one nucleic acid ligase and at least one nucleic acid polymerase,

c) incubating the mixture of step b), under conditions which allow said at least one nucleic acid ligase and at least one nucleic acid polymerase to be active,

d) contacting the mixture of step c) with at least one solid phase,

e) measuring total nucleic acid in said sample by measuring the total amount of said at least one detectable species or the amount of said at least one binding species bound to said solid phase, and

f) determining whether the total nucleic acid in said sample is higher or lower than a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg.

8. (3x Amended) A method as in claim 1, wherein said at least one nucleic acid polymerase is selected from the group consisting of Taq DNA polymerase, T4 DNA

polymerase, Klenow fragment, Pfu DNA polymerase, Exo- Pfu DNA polymerase, E. coli DNA polymerase I, Klenow fragment of DNA polymerase I, MMLV reverse transcriptase and AMV reverse transcriptase.

12. (2x Amended) A method as in claim 1, wherein said conditions comprise a solution with a pH between 5.5 and 9.5, a nucleotide triphosphate concentration between 1 pM and 10 mM, a Mg2+ concentration between 0.05 mM and 500 mM, and a reducing agent concentration between 0 and 500 mM, wherein the sum of the molarities is between 1 mM and 500 mM.

13. (3x Amended) A method as in claim 4, wherein said at least one ligase is selected from the group consisting of Pfu DNA ligase, T4 DNA ligase, Taq DNA ligase, T4 RNA ligase, and E. coli DNA ligase.

14. (Amended) A method as in claim 1, wherein said random primer is from 4 to 20 nucleotides in length.

15. (2x Amended) A method as in claim 14, wherein said at least one detectable is selected from the group consisting of biotin, nucleic acid sequence, nucleic acid base pairing linear polymer, fluorescent molecule, electrochemiluminescent molecule, radioactive molecule, peroxidase and alkaline phosphatase.

16. (Amended) A method as in claim 14, wherein said at least one binding species is selected from the group consisting of biotin, antigen, lectin, ligand, hormone, nucleic acid sequence, mimitope and nucleic acid base pairing linear polymer.

17. (Amended) A method as in claim 14, wherein said at least one nucleic acid polymerase is selected from the group consisting of Taq DNA polymerase, Klenow fragment (3'-5') of E. coli DNA polymerase I and Klenow fragment of DNA polymerase I.

18. (Amended) A method as in claim 14, wherein said at least one solid phase is selected from the group consisting of magnetic bead, plastic plate and polymer bead.

19. (2x Amended) A method as in claim 14, wherein said at least one nucleotide triphosphate is selected from the group consisting of dATP, dGTP, dCTP, dUTP, dTTP, 7-deaza dGTP, biotin-dATP, biotin-dCTP, biotin-dUTP, digoxigenin dUTP, digoxigenin UTP and biotin ddUTP.

20. (Amended) A method as in claim 14, wherein said random primer is 6-10 nucleotides in length.

21. (Amended) A method as in claim 14, wherein said conditions comprise those optimal for Klenow fragment of DNA polymerase I to synthesize DNA.

22. (2x Amended) A method as in claim 20, wherein said NTP is a dNTP.

23. (3x Amended) A method for determining [measuring] total nucleic acid in a sample, which comprises:

a) mixing at least one random primer at least 4 nucleotides in length having at least one detectable species, with a sample which may contain nucleic acid,

b) adding at least one nucleotide triphosphate having at least one binding species and optionally at least one second nucleotide triphosphate,

c) adding at least one nucleic acid polymerase,

d) incubating the mixture of step c), under conditions which allow said at least one nucleic acid polymerase to be active,

e) measuring total nucleic acid in said sample by measuring the total amount of said at least one detectable species or the amount of said at least one binding species, and

f) determining whether the total nucleic acid in said sample is higher or lower than a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg.

24. (3x Amended) A method for determining [measuring] total nucleic acid in a sample, which comprises:

a) mixing at least one random primer at least 4 nucleotides in length, having at least one binding species, with a sample which may contain nucleic acid,

b) adding at least one nucleotide triphosphate having at least one detectable species and optionally at least one second nucleotide triphosphate,

c) adding at least one nucleic acid polymerase,

d) incubating the mixture of step c), under conditions which allow said at least one nucleic acid polymerase to be active,

e) measuring total nucleic acid in said sample by measuring the total amount of said at least one detectable species or the amount of said at least one binding species, and

f) determining whether the total nucleic acid in said sample is higher or lower than a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg.

25. (3x Amended) A method for determining [measuring] total nucleic acid in a sample, which comprises:

a) mixing at least one random primer at least 4 nucleotides in length with a sample which may contain nucleic acid,

b) adding at least one nucleotide triphosphate having at least one binding moiety and optionally at least one second nucleotide triphosphate having at least one label and optionally at least one nucleotide triphosphate,

c) adding at least one nucleic acid polymerase,

d) incubating the mixture of step c), under conditions which allow said at least one nucleic acid polymerase to be active,

e) measuring total nucleic acid in said sample by measuring the total amount of said at least one label or the amount of said at least one binding moiety, and

f) determining whether the total nucleic acid in said sample is higher or lower than a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg.

26. (3x Amended) A method for determining [measuring] total nucleic acid in a sample, which comprises:

a) mixing at least one labeled random primer at least 4 nucleotides in length having at least one binding species and optionally at least one second random primer at least 4 nucleotides in length having at least one detectable species, with a sample nucleic acid,

b) adding at least one nucleic acid ligase,

c) incubating the mixture of step b), under conditions which allow said at least one nucleic acid ligase to be active,

d) measuring total nucleic acid in said sample by measuring the total amount of said at least one detectable species or the amount of said at least one binding species, and

e) determining whether the total nucleic acid in said sample is higher or lower than a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg.

27. (3x Amended) A method for determining [measuring] total nucleic acid in a sample, which comprises:

a) mixing at least one labeled random primer at least 4 nucleotides in length having at least one binding species and optionally at least one second random primer at least 4 nucleotides in length having at least one detectable species, with a sample which may contain nucleic acid,

b) adding at least one nucleic acid ligase and at least one nucleic acid polymerase,

c) adding at least one nucleotide triphosphate,

d) incubating the mixture of step c), under conditions which allow said at least one nucleic acid ligase and at least one nucleic acid polymerase to be active,

e) measuring total nucleic acid in said sample by measuring the total amount of said at least one detectable species or the amount of said at least one binding species, and

f) determining whether the total nucleic acid in said sample is higher or lower than a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg.

29. (2x Amended) A method as in claim 1, wherein said at least one nucleic acid polymerase is selected from the group consisting of Taq DNA polymerase, T4 DNA polymerase, Klenow fragment, Pfu DNA polymerase, Exo- Pfu DNA polymerase, E. coli DNA polymerase I, Klenow fragment of DNA polymerase I, MMLV reverse transcriptase and AMV reverse transcriptase.

33. (3x Amended) A method as in claim 26, wherein said at least one ligase is selected from the group consisting of Pfu DNA ligase, T4 DNA ligase, Taq DNA ligase, T4 RNA ligase, and E. coli DNA ligase.

34. (2x Amended) A kit comprising:

a) a vial containing at least one random primer at least 4 nucleotides in length having at least one detectable species, and containing at least one NTP having at least one binding species and optionally at least one NTP,

b) a vial containing at least one nucleic acid polymerase, [and]

c) a vial containing at least one solid phase, and

d) a vial containing a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg total nucleic acid.

35. (2x Amended) A kit as in claim 34, where[ ]in component a) consists of a vial containing at least one random primer at least 4 nucleotides in length having at least one species, and a vial containing at least one NTP having at least one binding detectable species and optionally at least one NTP.

36. (2x Amended) A kit comprising:

a) a vial containing at least one random primer at least 4 nucleotides in length having at least one binding species, and containing at least one NTP having at least one detectable species and optionally at least one NTP,

b) a vial containing at least one nucleic acid polymerase, [and]

c) a vial containing at least one solid phase, and

d) a vial containing a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg total nucleic acid.

37. (2x Amended) A kit as in claim 36, where[ ]in component a) consists of a vial containing at least one random primer at least 4 nucleotides in length having at least one binding species, and a vial containing at least one NTP having at least one detectable species and optionally at least one NTP.

38. (2x Amended) A method for determining [measuring] total nucleic acid in a sample, which comprises:

a) mixing at least one random primer at least 4 nucleotides in length having at least one first label, with a sample nucleic acid,

b) adding at least one nucleotide triphosphate having at least one second label and optionally at least one second nucleotide triphosphate,

c) adding at least one nucleic acid polymerase,

d) incubating the mixture of step c), under conditions which allow said at least one nucleic acid polymerase to be active,

e) measuring total nucleic acid in said sample by measuring the total amount of said at least one first label or the amount of said at least one second label, and

f) determining whether the total nucleic acid in said sample is higher or lower than a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg.

39. (2x Amended) A method for determining [measuring] total nucleic acid in a sample, which comprises:

a) mixing at least one labeled random primer at least 4 nucleotides in length having at least one first label species and optionally at least one second random primer at least 4 nucleotides in length having at least one second label, with a sample which may contain nucleic acid,

b) adding at least one nucleic acid ligase,

c) incubating the mixture of step b), under conditions which allow said at least one nucleic acid ligase to be active,

d) measuring total nucleic acid in said sample by measuring the total amount of said at least one first label or the amount of said at least one second label, and

e) determining whether the total nucleic acid in said sample is higher or lower than a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg.

40. (2x Amended) A method for determining [measuring] total nucleic acid in a sample, which comprises:

a) mixing at least one labeled random primer at least 4 nucleotides in length having at least one first label and optionally at least one second random primer at least 4 nucleotides in length having at least one second label, with a sample which may contain nucleic acid,

b) adding at least one nucleic acid ligase and at least one nucleic acid polymerase,

c) adding at least one nucleotide triphosphate,

d) incubating the mixture of step c), under conditions which allow said at least one nucleic acid ligase and at least one nucleic acid polymerase to be active,

e) measuring total nucleic acid in said sample by measuring the total amount of said at least one first label or the amount of said at least one second label, and

f) determining whether the total nucleic acid in said sample is higher or lower than a threshold amount of contamination, wherein the threshold amount of contamination is equal to or less than 100 pg.